Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4853–4857, July 1989
Biochemistry

cAMP stimulates transcription of the β_2-adrenergic receptor gene in response to short-term agonist exposure

(transcriptional activation/second messenger/epinephrine/transfection/down-regulation)

SHEILA COLLINS*, MICHEL BOUVIER*, MARK A. BOLANOWSKI*†, MARC G. CARON*, AND ROBERT J. LEFKOWITZ*

*Howard Hughes Medical Institute, Departments of Medicine, Biochemistry, and Cell Biology, Duke University Medical Center, Durham, NC 27710; and †Molecular Genetics and Mammalian Biology Group, Biological Science Department, Monsanto, Saint Louis, MO 63198

Contributed by Robert J. Lefkowitz, March 27, 1989

ABSTRACT **In addition to conveying cellular responses to an effector molecule, receptors are often themselves regulated by their effectors. We have demonstrated that epinephrine modulates both the rate of transcription of the β_2-adrenergic receptor (β_2AR) gene and the steady-state level of β_2AR mRNA in DDT$_1$MF-2 cells. Short-term (30 min) exposure to epinephrine (100 nM) stimulates the rate of β_2AR gene transcription, resulting in a 3- to 4-fold increase in steady-state β_2AR mRNA levels. These effects are mimicked by 1 mM N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (Bt$_2$cAMP) or forskolin but not by phorbol esters. The half-life of the β_2AR mRNA after addition of actinomycin D (46.7 ± 10.2 min; mean ± SEM; n = 5) remained unchanged after 30 min of epinephrine treatment (46.8 ± 10.6 min; mean ± SEM; n = 4), indicating that a change in transcription rate is the predominant factor responsible for the increase of β_2AR mRNA. Whereas brief exposure to epinephrine or Bt$_2$cAMP does not significantly affect the total number of cellular β_2ARs (assessed by ligand binding), continued exposure results in a gradual decline in β_2AR number to ≈20% (epinephrine) or ≈45% (Bt$_2$cAMP) of the levels in control cells by 24 hr. Similar decreases in agonist-stimulated adenylyl cyclase activity are observed. This loss of receptors with prolonged agonist exposure is accompanied by a 50% reduction in β_2AR mRNA. Transfection of the β_2AR promoter region cloned onto a reporter gene (bacterial chloramphenicol acetyltransferase) allowed demonstration of a 2- to 4-fold induction of transcription by agents that elevate cAMP levels, such as forskolin or phosphodiesterase inhibitors. These results establish the presence of elements within the proximal promoter region of the β_2AR gene responsible for the transcriptional enhancing activity of cAMP and demonstrate that β_2AR gene expression is regulated by a type of feedback mechanism involving the second messenger cAMP.**

Receptor activation can stimulate or inhibit a variety of cellular processes from basic metabolic activities to certain highly specialized functions in differentiated tissues. Although receptors serve as regulators of these cellular activities, they are themselves subject to considerable regulation. For most receptors, stimulation is followed by a period of reduced responsiveness or "desensitization" (1), which usually involves some combination of receptor phosphorylation, sequestration or loss of receptor number, and uncoupling of the receptor from the effector (2, 3). In addition to this regulation at the protein level, the expression of the receptor gene itself can be modulated as a consequence of activation. In several cases (4–6) mRNA levels for the receptor are rapidly reduced following stimulation, thereby further contributing to the loss in receptor number and responsiveness, whereas for other receptors (7, 8) expression is elevated by processes that involve both transcriptional and posttranscriptional components. In addition, complex forms of regulation displaying early transient increases followed by a down-regulation of expression over time have also been observed (9, 10). Clearly, this diversity of receptor regulation must depend upon different cell signaling pathways which themselves serve to regulate gene expression.

The family of G-protein-coupled receptors regulate the intracellular concentrations of important second messenger molecules such as cAMP, calcium, and diacylglycerol. The β_2-adrenergic receptor (β_2AR) is a prototypic member of the G-protein coupled receptor family that is linked to the stimulation of adenylyl cyclase (11, 12). The β_2AR has been shown to be a substrate for cAMP-dependent protein kinase, resulting in a form of feedback attenuation of responsiveness (13). In addition to these and other diverse regulatory and metabolic effects of cAMP mediated through cAMP-dependent protein kinase, cAMP has also been shown to enhance the expression of many genes (14). In most cases this increased gene expression is due to transcriptional activation, although in some cases mRNA stability is also affected (15–17). The transcriptional enhancing properties of cAMP are mediated by distinct promoter elements within the target gene, which are termed cAMP response elements (CREs). Most CREs contain a variation of the palindromic sequence motif TGACGTCA, which is recognized by specific phosphoprotein transcription factors, some of which have been purified (18) and recently cloned (19, 20). It is interesting to note that many genes regulated by cAMP encode protein products which are themselves regulated or whose secretion is evoked by hormones that elevate intracellular levels of cAMP (15–17, 21–24). Inspection of the 5' flanking region of the β_2AR gene from both hamster and human reveals the presence of putative CREs, suggesting that it, too, may be subject to regulation by cAMP at both the protein and the transcriptional level.

Recently it has been reported that down-regulation of β_2AR following long-term agonist exposure is accompanied by a decrease in β_2AR mRNA (25). In the studies reported here, we describe changes in both β_2AR gene transcription in response to short-term agonist exposure as well as down-regulation of β_2AR mRNA levels resulting from more prolonged exposure. Our results suggest a form of feedback regulation at the level of receptor gene expression that is mediated by the second messenger cAMP.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: β_2AR, β_2-adrenergic receptor; CAT, chloramphenicol acetyltransferase; CRE, cAMP response element; CYP, cyanopindolol; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; IBMX, isobutylmethylxanthine; PMA, phorbol 12-myristate 13-acetate; Bt$_2$cAMP, N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate; EGF, epidermal growth factor.

MATERIALS AND METHODS

Cell Cultures, Transfections, and Chloramphenicol Acetyltransferase (CAT) Assays. DDT_1MF-2 hamster smooth muscle cells were grown in suspension culture as described (26). Experiments were initiated at a cell density of $\approx 2.2 \times 10^5$ cells per ml. Catecholamines were added to cells in the presence of superoxide dismutase and catalase (1 μg/ml final concentration) to prevent catecholamine oxidation (27).

Monolayer cultures of rat C6 glioma cells were grown in Dulbecco's modified Eagle's medium/Ham's F-10 medium (50:50) supplemented with 10% fetal bovine serum. Transfections of C6 glioma cells were performed with calcium phosphate precipitates (28) containing 10 μg of test plasmid DNA. Cells were incubated with the precipitate for 4 hr, treated with 15% glycerol for 2 min, and supplemented with fresh medium. After 24 hr, forskolin (in ethanol), isobutylmethylxanthine [IBMX; in dimethylformamide (DMF)], or phorbol 12-myristate 13-acetate [PMA; in dimethyl sulfoxide (DMSO)] was added to cells at a final concentration of 25 μM, 0.25 mM, or 100 nM, respectively. Cell extracts were prepared 15–18 hr after the addition of drugs by freezing-thawing as described (29). Protein concentrations of the extracts were determined by the method of Bradford (30). CAT assays, containing 50–100 μg of protein, 0.3 μCi of [^{14}C]chloramphenicol (54 Ci/mmol; 1 Ci = 37 GBq; Amersham), and 1.1 mM acetyl-CoA in a final volume of 150 μl, were incubated for 60 min at 37°C, extracted with ethyl acetate, and chromatographed (29).

Plasmid Constructions. The vector for all CAT constructs was p-L-CAT-2, which was obtained from Russell Kaufman (Duke University). For the β_2AR promoter clone p1.4, a 1440-base-pair (bp) *Hinf* I fragment from nucleotide −1355 to +85 relative to the transcription initiation site (31) was isolated by gel electrophoresis. Overhanging 5′ ends were filled in with the Klenow fragment of DNA polymerase I and excess dNTPs and were ligated into the *Sma* I site in the polylinker of the CAT-containing vector p-L-CAT-2. Similarly, for β_2AR clone p0.3, a 305-bp *Alu* I fragment from nucleotide −235 to +70 was isolated and ligated into the *Sma* I site of p-L-CAT-2. The clones were verified with respect to their orientation and integrity by restriction mapping and DNA sequencing.

Radioligand Binding and Adenylyl Cyclase Assays. Preparation of plasma membranes, binding of the βAR-specific ligand ^{125}I-labeled cyanopindolol (^{125}I-CYP), and adenylyl cyclase assays were performed as outlined (26).

RNA Analysis. Total cellular RNA was isolated from DDT_1MF-2 cells by the cesium chloride gradient method (32). Following denaturation by glyoxalation and fractionation by electrophoresis, the RNA was immobilized on nylon membranes and hybridized to β_2AR and actin cDNA probes, all as previously detailed (26).

For nuclear run-off transcription assays, DDT_1MF-2 cells were harvested by centrifugation and washed in a buffer containing 5 mM Tris (pH 7.5) and 1.5 mM $MgCl_2$. Nuclei were prepared, counted, and used immediately without freezing (1–3 $\times 10^7$ per incubation). Subsequent steps of [^{32}P]RNA isolation and hybridization were performed exactly as described (26). Following autoradiography, radiolabeled spots were cut out and assayed for radioactivity. The synthesis rates were calculated from [^{32}P]RNA bound to the specific cDNAs minus radioactivity bound to the pGEM control and corrected for the amount of input labeled RNA in millions of cpm. The parts per million (ppm) for β_2AR synthesis were normalized to β-actin ppm values.

RESULTS

Following the addition of 100 nM epinephrine to DDT_1MF-2 cells, there was a rapid increase in the steady-state level of β_2AR mRNA. This 3- to 4-fold increase in the level of β_2AR mRNA was evident 30–60 min after addition of the hormone (Fig. 1). No change in actin mRNA was apparent. The major hybridizing band at 2.2 kilobases (kb) and the minor band at 1.8 kb have been observed previously (26) and most likely result from the use of two poly(A) signals ≈400 bp apart. Since these two mRNA species varied in parallel, they were analyzed together. Over prolonged exposure, a steady decline in β_2AR mRNA levels is evident such that by 24 hr, levels are reduced >50% below the pretreatment level.

Despite the stimulation of β_2AR mRNA levels, receptor levels (Fig. 2), as measured by the binding of the antagonist ^{125}I-CYP, were not changed during the first few hours. In some experiments slightly elevated levels of receptor were found 30 min after addition of the catecholamine, but this was not statistically significant. Prolonged exposure led to the typical agonist-promoted down-regulation of receptors (1) to ≈20% of pretreatment levels within 24–28 hr. Results from whole cell binding experiments were essentially identical to the results obtained in plasma membranes (M.B. and S.C., unpublished observations). Cells that received only the medium vehicle displayed no changes in receptor number or mRNA accumulation (not shown).

The role of cAMP in the transient stimulation of β_2AR mRNA levels was investigated by treating cells with N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP) (1 mM) and the phosphodiesterase inhibitor IBMX (0.25 mM). β_2AR mRNA levels were regulated by Bt_2cAMP in a fashion similar to that induced by epinephrine (Fig. 3*A*). A 2.5- to 3-fold increase in β_2AR mRNA was observed 1.5 hr after exposure to the drug. This enhancement rapidly returned to control levels, whereupon there was a further decline to ≈30% of initial levels. By 24 hr, transcript levels began to recover. Again, in contrast to the stimulation of β_2AR mRNA, the number of β_2ARs was unchanged during the first few hours, with an ultimate decline to 45% of pretreatment levels (Fig. 3*B*) occurring by 24 hr.

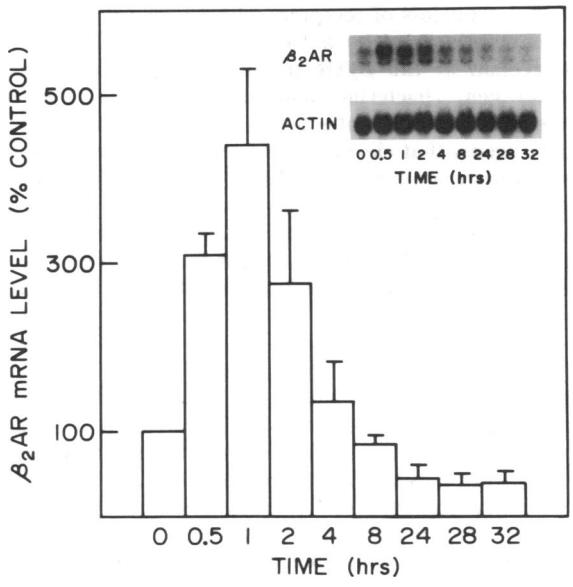

FIG. 1. Time course of changes in β_2AR mRNA levels after addition of epinephrine. Total cellular RNA was prepared from DDT_1MF-2 cells immediately before (0 hr) or at the indicated times after addition of epinephrine (100 nM). Northern blots were prepared, and the levels of β_2AR and actin mRNAs were analyzed by scanning the autoradiograms with a laser densitometer. β_2AR mRNA levels (with 0 hr defined as 100%) are expressed relative to actin mRNA, as actin expression is unaffected by epinephrine. The data shown are the average ± SEM of three experiments. (*Inset*) Representative Northern blot.

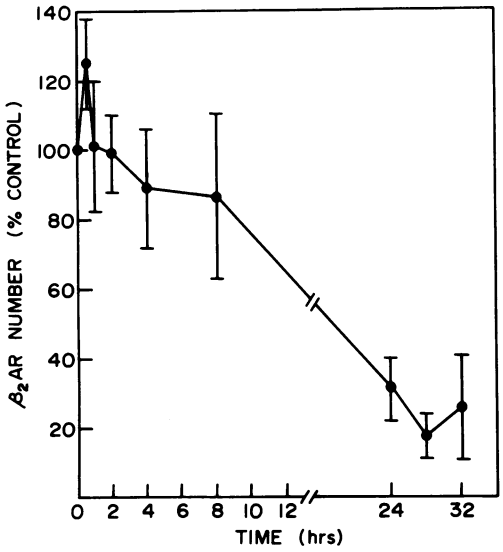

FIG. 2. Time course of changes in β_2AR number in plasma membranes from DDT$_1$MF-2 cells after addition of epinephrine. Cells were harvested immediately before (0 hr) or at the indicated times after addition of epinephrine (100 nM). Plasma membranes were prepared and β_2AR density was determined by binding of the antagonist ^{125}I-CYP. The data shown are the average ± SEM of three experiments.

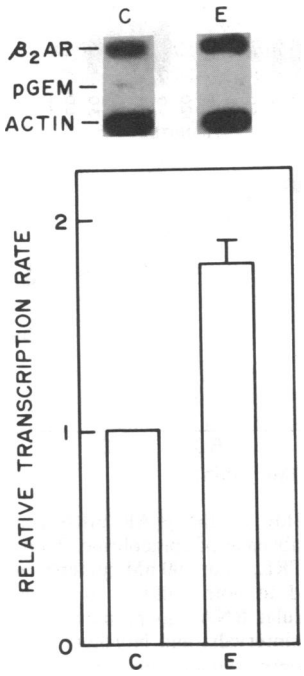

FIG. 4. Relative transcription rate of the β_2AR gene in control (C) or epinephrine-treated (E) cells assessed by nuclear run-off transcription assay. Nuclei were prepared 30 min after addition of medium vehicle or epinephrine (100 nM) and incubated in the presence of 200 μCi of [α-^{32}P]UTP. The [^{32}P]RNA was isolated and hybridized to plasmid DNAs (10 μg per slot) containing β_2AR or actin cDNA inserts or without insert (pGEM). Following autoradiography, radiolabeled spots were cut out and assayed for radioactivity. Background hybridization to pGEM was subtracted from values for β_2AR and actin. The fold increase in β_2AR transcription (1.77 ± 0.11; mean ± SEM; $n = 3$; $P < 0.01$) was essentially identical whether determined by laser scanning or scintillation spectroscopy and was comparable to the increase in steady-state β_2AR mRNA (2.35 ± 0.66; mean ± SEM; $n = 3$; $P < 0.025$) determined from Northern blots.

The induction of β_2AR mRNA levels was specific to β_2AR activation and cAMP generation. The effect of epinephrine was mediated through the β_2AR rather than the α_1-adrenergic receptor (α_1AR) on these cells, since propranolol, a β_2AR antagonist, blocked the increase in β_2AR mRNA, whereas phentolamine, an α_1AR-specific antagonist, did not. As expected, isoproterenol, a βAR-specific agonist, increased β_2AR mRNA levels as early as 10 min after its addition (not shown).

Changes in the steady-state level of expression of a gene result from alterations in either the rate of transcription, the rate of degradation of the message, or a combination of the two processes. For several genes, there is evidence for cAMP-mediated changes in both of these pathways (15–17, 22). We therefore investigated the mechanisms underlying the cAMP-induced elevation of β_2AR mRNA levels that occurs soon after exposure to agents that elevate cAMP levels. Nuclear run-off transcription assays were employed to monitor changes in the rate of transcription of the β_2AR and actin genes in DDT$_1$MF-2 cells treated with 100 nM epinephrine or medium vehicle alone. In addition, total RNA was prepared from aliquots of cells so that the transcription rate measured in a given experiment could be compared directly to the change in steady-state message levels. The results of this series of experiments are depicted in Fig. 4. The rate of β_2AR gene transcription was nearly doubled in nuclei from cells pretreated with epinephrine for 30 min, whereas the rate of actin gene transcription was essentially unchanged. The enhancement of β_2AR gene transcription observed (1.77 ± 0.11; mean ± SEM; $n = 3$) is in reasonable agreement with the fold increase in the steady-state levels of β_2AR mRNA in these experiments (2.35 ± 0.66; mean ± SEM; $n = 3$) determined by Northern blotting. Thus the rapid increase in β_2AR mRNA levels induced by cAMP is the result of enhanced transcription of the β_2AR gene.

This conclusion was supported by additional experiments that examined the half-life of the β_2AR mRNA. After a 30-min incubation with either 100 nM epinephrine or medium alone, actinomycin D was added (1 μg/ml final concentration), and the disappearance of steady-state β_2AR mRNA was monitored at timed intervals (Fig. 5). Although β_2AR transcripts were more abundant in cells treated with epinephrine for 30 min than in the untreated cells, the rate at which this mRNA decayed ($t_{1/2}$ = 46.8 ± 10.6 min; mean ± SEM; $n = 4$) was unchanged relative to the untreated control cells ($t_{1/2}$ = 46.7

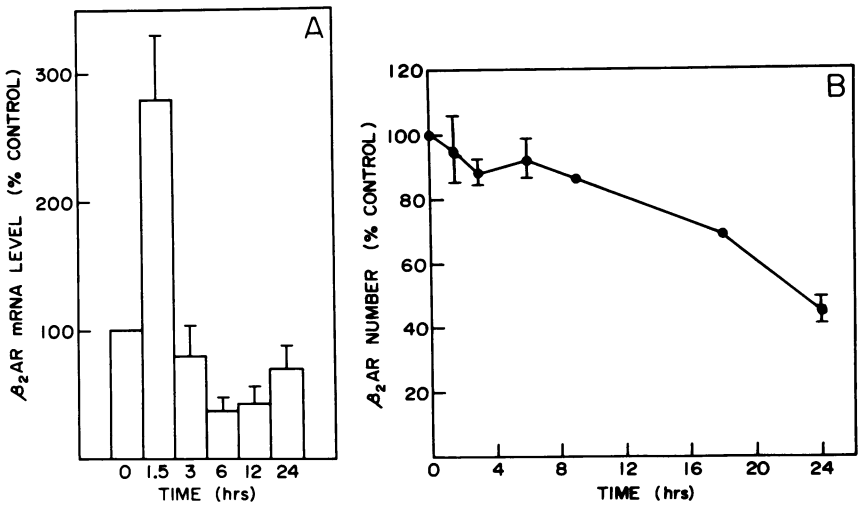

FIG. 3. Time course of changes in β_2AR mRNA levels and receptor number induced by Bt$_2$cAMP. (*A*) β_2AR mRNA levels. Northern blots were prepared from total cellular RNA after addition of Bt$_2$cAMP (1 mM) and IBMX (0.25 mM). Autoradiograms were analyzed by scanning laser densitometry, and β_2AR mRNA levels are expressed relative to actin mRNA (with 0 hr defined as 100%). The data shown are the average ± SEM of three experiments. (*B*) β_2AR density in plasma membranes was assessed by the binding of ^{125}I-CYP. The results presented are the average of three experiments.

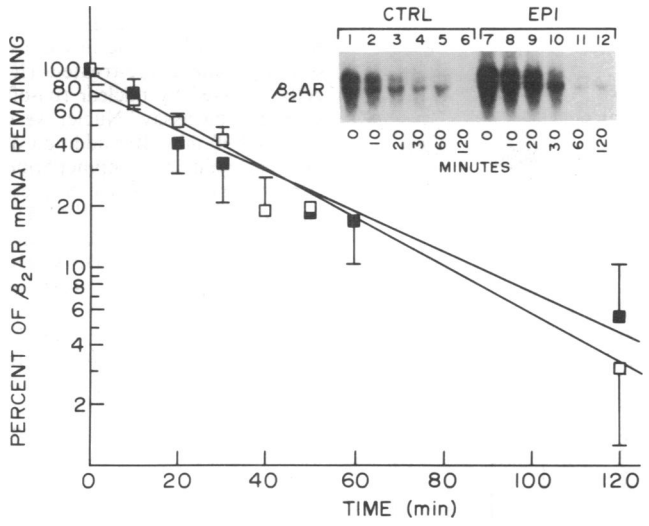

FIG. 5. Estimation of the half-life of the β_2AR mRNA in DDT$_1$MF-2 cells in the presence or absence of epinephrine. Thirty minutes after addition of medium (CTRL, □) or 100 nM epinephrine (EPI, ■) actinomycin D was added to both cultures to a final concentration of 1 μg/ml. Total cellular RNA was prepared from aliquots of cells collected at 10-min intervals, and Northern blots were prepared. The autoradiograms were scanned and the data were fit by least-squares linear regression analysis. The slopes for the two groups were compared by a two-tailed t test. This analysis was confined to the more abundant upper hybridizing band only since it was not always possible to accurately measure the lower band due to background hybridization in that region. In a few experiments where a clear analysis was possible, the $t_{1/2}$ of the lower band was ≈80 min. (*Inset*) Representative Northern blot of β_2AR mRNA.

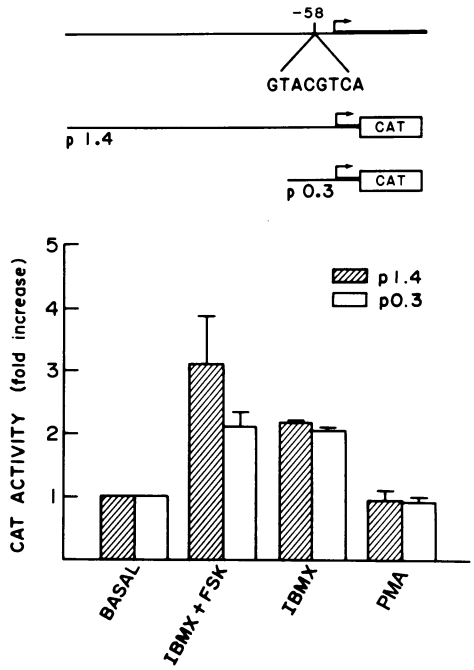

FIG. 6. cAMP stimulation of CAT enzyme activity in C6 glioma cells transfected with β_2AR 5′ flanking sequences: Schematic diagram of the β_2AR gene indicating the location and sequence of the putative CRE at −58 bp relative to the start site of transcription (arrow). C6 glioma cells were transfected with CAT plasmid DNAs containing 1335 bp (p1.4) or 235 bp (p0.3) of human β_2AR 5′ flanking DNA (10 μg of DNA per 100-mm dish) by the calcium phosphate precipitation method. CAT enzyme activity was measured in cell extracts 18–20 hr after addition of forskolin (FSK) (25 mM), IBMX (0.25 mM), or PMA (100 nM). The data shown are the average of four separate experiments, each performed in duplicate. Slight differences in CAT activity observed between p1.4 and p0.3 in response to IBMX plus forskolin were not significant by a t test ($P < 0.05$).

± 10.2; mean ± SEM; n = 5). The 1.8-kb mRNA appeared to decay with slower kinetics, such that at later times it became the predominant species.

For many genes that are transcriptionally regulated by cAMP, the 5′ flanking promoter regions are necessary and sufficient to carry out this activity (14). Within these regions specific sequences have been identified as critical for this function and are termed CREs. In the promoter regions of the β_2AR gene from hamster and human, sequences that conform to the CRE consensus are present between −50 and −60 from the start site of transcription. We therefore constructed promoter–reporter gene fusions between the 5′ flanking region of the human β_2AR and the coding sequences of the bacterial CAT gene. The test plasmids contained β_2AR promoter sequences extending −1355 (p1.4) or −235 (p0.3) bp upstream from the transcription start site. These constructs were introduced by DNA-mediated gene transfer (28) into rat C6 glioma cells. Fig. 6 demonstrates that CAT activity of both constructs is enhanced 2- to 4-fold over basal expression by forskolin plus IBMX or IBMX alone but not by PMA. Forskolin also stimulates CAT activity when these constructs are transfected into a human T-cell line (S.C. and Dean Ballard, unpublished observations). These results establish that the promoter region of the β_2AR contains elements responsible for the transcriptional enhancing activity of cAMP as observed by nuclear run-off transcription assays.

DISCUSSION

Our results document a transient β-agonist stimulated, cAMP-mediated increase in the steady-state level of β_2AR mRNA resulting from an increase in the rate of transcription of the β_2AR gene. A corresponding increase in the number of cellular β_2ARs was not detected, however. This likely represents the net result of simultaneous disappearance of receptors (e.g., by sequestration and degradation) and synthesis of new receptors. This would preclude detection of a significant rise in β_2AR by ligand-binding techniques. Consistent with this formulation, in cells transfected with a β_2AR cDNA lacking the 5′ regulatory regions, the rates of agonist- or cAMP-induced down-regulation of the β_2AR are considerably faster than those observed here (M.B., S.C., and Paul Campbell, unpublished observations). A similar situation has been encountered in earlier studies of epidermal growth factor (EGF) receptor regulation following agonist exposure (8). In this case, ligand-bound EGF receptor internalization and degradation were occurring while receptor mRNA levels were increasing, thus preventing detectable increases in EGF receptor number. Analogous processes may be occurring for the β_2AR.

The EGF receptor paradigm highlights an important feature of signal transduction in general: that cells maintain stringent control over hormonal responsiveness by regulating multiple steps in the signal transduction pathway. Mechanisms exist to regulate both receptor–effector coupling and receptor number. For the β_2AR, within minutes of agonist exposure, receptors are rapidly uncoupled and sequestered from their effector, adenylyl cyclase, with receptor phosphorylation participating in this process (1–3). From a physiological perspective, most neural and endocrine tissues on which these receptors reside receive brief, periodic bursts of hormone. In these cells the signal transduction apparatus must be flexible enough to respond to repeated stimulation. Transient stimulation of β_2AR synthesis, mediated by the second messenger cAMP, may be an important element in maintaining this cellular responsiveness.

Most genes that are transcriptionally regulated by cAMP have relatively short-lived mRNAs (minutes vs. hours; ref. 14). Our data estimate the half-life for the β_2AR mRNA in DDT_1MF-2 cells to be ≈45 min, consistent with this regulatory paradigm. This estimate, however, differs dramatically from the value of 12 hr recently reported by Hadcock and Malbon (33). The reasons for this discrepancy are not clear but might include the following: (*i*) our DDT_1MF-2 cells were grown as a suspension culture, whereas Hadcock and Malbon maintained this cell line in monolayer culture, and (*ii*) different methods of RNA analysis were used (Northern blotting with <25 μg of total cellular RNA in our case vs. solution hybridization and filter binding by Hadcock and Malbon with 150 μg of RNA).

Quantitatively, the effect of cAMP on the increase in the transcription rate of the β_2AR gene is similar to that observed for other cAMP-regulated genes such as the glycoprotein hormone α-subunit gene (22), phospho*enol*pyruvate carboxykinase (16), tyrosine aminotransferase (17), and lactate dehydrogenase (15). The presence of CREs in the promoters of these genes (21–24) has been shown to be responsible for this stimulation. For the β_2AR gene, transcriptional enhancement may also be mediated by a CRE within the promoter region. The results from transient transfection of β_2AR promoter-CAT constructs into C6 glioma cells, which yield a 2- to 4-fold stimulation of CAT activity in response to forskolin and IBMX, fully support the results of nuclear run-off transcription assays. The extent of induction by these agents is similar for both constructs (p1.4 and p0.3), indicating that the sequences responsible for transcriptional enhancement are present within the proximal 235 bp of β_2AR 5′ flanking DNA. As shown in Fig. 6, the sequence GTACGTCA appears within this region (−58) in both the human and hamster β_2AR genes and displays close homology to these other CREs (14, 21–24). More detailed study of this region by mutational analysis and other approaches aimed at identifying sequences that may be specifically recognized by cAMP-regulated nuclear factors (19, 20) should allow the characterization of this element more precisely.

We have not focused here on the observation, made both in these studies and by others (25), that prolonged exposure of cells to agonist results in a decrease in β_2AR mRNA. This area, however, also requires further studies to establish a comprehensive understanding of the mechanisms of desensitization and down-regulation of the β_2AR. Recent studies (25) have shown that cAMP is involved in this down-regulation of β_2AR mRNA levels, but as yet no mechanism has been described to explain this observation. For several other cAMP-regulated genes that display early and transient accumulation of mRNA, dynamic changes in both the transcription rate and mRNA stability occur. For example, in the case of the tyrosine aminotransferase gene in particular, temporally distinct changes in the transcription rate and in the turnover of tyrosine aminotransferase mRNA have been observed, depending upon the duration of exposure to cAMP analogues (17). This type of complex regulation may also exist for the β_2AR gene during down-regulation. Recently, we have obtained evidence from cells transfected with a human β_2AR cDNA for cAMP-mediated changes in β_2AR mRNA levels that are distinct from transcriptional effects (M.B. and S.C., unpublished observations), suggesting alterations in mRNA turnover.

Our results establish that the expression of the β_2AR gene is regulated by its second messenger, cAMP. Like other cAMP-regulated genes, elements within the 5′ flanking region of the gene mediate this transcriptional enhancement by cAMP. To our knowledge, this form of regulation, which may represent a physiologically important feedback mechanism for maintaining hormonal responsiveness, has not been found previously for an adenylyl cyclase-coupled receptor.

We thank Dr. Russell Kaufman for the p-L-CAT-2, Sherry Brilliant and Sabrina Taylor Exum for their superb technical assistance, Grace Irons for help with transfections, and Oded Herbsman for his contributions to the early phases of this project. We also thank Pamela Mellon, Susanna Cotecchia, and William C. Wetsel for their advice and helpful discussions, Richard W. Hanson for his critical review of the manuscript, Lynda Frejlach for artwork, and Donna Addison for secretarial assistance.

1. Sibley, D. R. & Lefkowitz, R. J. (1985) *Nature (London)* **317,** 124–129.
2. Bouvier, M., Hausdorff, W. P., De Blasi, A., O'Dowd, B. F., Kobilka, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **333,** 370–373.
3. Benovic, J. L., Bouvier, M., Caron, M. G. & Lefkowitz, R. J. (1988) *Annu. Rev. Cell Biol.* **4,** 405–428.
4. Oron, Y., Straub, R. E., Traktman, P. & Gershengorn, M. C. (1987) *Science* **238,** 1406–1408.
5. Russel, D. W., Yamamoto, T., Schneider, W. J., Slaughter, C. J., Brown, M. S. & Goldstein, J. L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7501–7505.
6. Wei, L. L., Krett, N. L., Francis, M. D., Gordon, D. F., Wood, W. M., O'Malley, B. W. & Horwitz, K. B. (1988) *Mol. Endocrinol.* **2,** 62–72.
7. McDonnell, D. P., Mangelsdorf, D. J., Wesley, J., Haussler, M. R. & O'Malley, B. (1988) *Science* **235,** 1214–1217.
8. Clark, A. J. L., Ishii, S., Richert, N., Merlino, G. T. & Pastan, I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8374–8378.
9. Okret, S., Poellinger, L., Dong, Y. & Gustafsson, J.-A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5899–5903.
10. Dong, Y., Poellinger, L., Gustafsson, J.-A. & Okret, S. (1988) *Mol. Endocrinol.* **2,** 1256–1264.
11. Lefkowitz, R. J. & Caron, M. G. (1988) *J. Biol. Chem.* **263,** 4993–4996.
12. Levitski, A. (1988) *Science* **241,** 800–806.
13. Benovic, J. L., Pike, L. J., Cerione, R. A., Staniszewski, C., Yoshimasa, T., Codina, J., Caron, M. G. & Lefkowitz, R. J. (1985) *J. Biol. Chem.* **260,** 7094–7101.
14. Roesler, W. J., Vandenbark, G. R. & Hanson, R. W. (1988) *J. Biol. Chem.* **263,** 9063–9066.
15. Jungmann, R. A., Kelley, D. C., Miles, M. F. & Milkowski, D. M. (1983) *J. Biol. Chem.* **258,** 5312–5318.
16. Hod, Y. & Hanson, R. W. (1988) *J. Biol. Chem.* **263,** 7747–7752.
17. Smith, J. D. & Liu, A. Y.-C. (1988) *EMBO J.* **7,** 3711–3716.
18. Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H., III, & Montminy, M. R. (1988) *Nature (London)* **334,** 494–498.
19. Hoeffler, J. P., Meyer, T. E., Yun, Y., Jameson, J. L. & Habener, J. F. (1988) *Science* **242,** 1430–1433.
20. Gonzalez, G. A., Yamamoto, K. K., Fischer, W. H., Karr, D., Menzel, P., Biggs, W. H., III, Vale, W. W. & Montminy, M. R. (1989) *Nature (London)* **337,** 749–752.
21. Fink, J. S., Verhave, M., Kasper, S., Tsukada, T., Mandel, G. & Goodman, R. H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6662–6666.
22. Jameson, J. L., Jaffe, R. C., Gleason, S. L. & Habener, J. F. (1986) *Endocrinology* **119,** 2560–2567.
23. Bokar, J. A., Roesler, W. J., Vandenbark, G. R., Kaetzel, D. M., Hanson, R. W. & Nilson, J. H. (1988) *J. Biol. Chem.* **263,** 19740–19747.
24. Comb, M., Birnberg, N. C., Seasholtz, A., Herbert, E. & Goodman, H. W. (1986) *Nature (London)* **323,** 353–356.
25. Hadcock, J. R. & Malbon, C. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5021–5025.
26. Collins, S., Caron, M. G. & Lefkowitz, R. J. (1988) *J. Biol. Chem.* **263,** 9067–9070.
27. Mahan, L. C. & Insel, P. A. (1984) *Anal. Biochem.* **136,** 208–216.
28. Mellon, P. L., Parker, V., Gluzman, Y. & Maniatis, T. (1981) *Cell* **27,** 279–288.
29. Gorman, C. M., Moffat, L. F., Rowe, M. E. & Goodman, H. M. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
30. Bradford, M. M. (1973) *Anal. Biochem.* **72,** 248–254.
31. Kobilka, B. K., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Dixon, R. A. F., Keller, P., Caron, M. G. & Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262,** 7321–7327.
32. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
33. Hadcock, J. R. & Malbon, C. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8415–8419.